REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-33 are in this case. Claims 1-17, and 29-33 have been withdrawn from consideration. Claims 18-28 have been rejected. Claims 18 and 28 have now been amended.

Specification

The Examiner has required correction of informality in reference to ROSA mice, on pages 28, 38 and 39. The appropriate paragraphs have been amended to recite "ROSA" mice.

35 U.S.C. § 112, First paragraph Rejections

The Examiner has rejected claims 18-28 under 35 U.S.C. 112, first paragraph, for lack of enablement. The Examiner's rejections are respectfully traversed. Claims 18 and 28 have now been amended.

The Examiner has stated that claims 18-28, while being enabling for a method of generating an artificial microorgan using a devitalized, acellular three dimensional scaffold and seeding the scaffold with pluripotent cells, does not reasonably provide enablement for a method of generating such an artificial microorgan using a synthetic scaffold and seeding with differentiated cells.

Applicant wishes to point out that the present invention teaches the generation of microorgans of defined, critical dimensions, prepared from tissue explants, devitalized tissue explants and from synthetic scaffolds:

> "...cells can be used to seed micro-organ sized scaffolds, thus generating "synthetic" micro-organs. Such scaffolds can be synthetic polymeric scaffolds which are sized and configured such that when populated with cells, the cells positioned deepest within the scaffold are at least about 100 micrometers and not more than about 225 micrometers away from the cells positioned at a nearest surface formed on the scaffold."(Page 24, lines 6-12).

The use of polymers, such as the collagen or polyglycolic acid fiber matrices cited by the Examiner, for generation of scaffolds suitable for repopulation by cultured cells was well known in the art at the time of the invention (see, for example US Patent No. 5,855,610). While provision of suitable growth and other factors important to the desired tissue type of such a synthetic microorgan is similarly taught in the instant application, (see page 24, line 17 to page 25, line 13) the present invention clearly teaches that the tissue type and function of a sythetic microorgan prepared from collagen or polyglycolic acid fibres will be determined by the interplay of factors: culture conditions, context of implatation, seeded cell type, and the provision of tissue-specific factors, such as growth factors (e.g. TGF-beta). Thus, while the properties of synthetic microorgans may differ from those of microorgans prepared from acellular devitalized tissue explants, it is Applicant's strong opinion that the the critical dimensions unique to such synthetic microorgans of the present invention are clear to one of ordinary skill in the art in possession of the instant specification (see Table 1, page 21), and the preparation thereof would not require undue experimentation.

The abovementioned not withstanding, in order to further define the method of the present invention, Applicant has elected to amend claim 18 to include the limitation of "providing a devitalized, acellular three dimensional scaffold..." for which the instant specification is clearly enabling (see Materials and Experimental Methods, page 28, and Examiner's comments, page 2 of Official Action Sumary, June 16, 2004). Thus, independent claim 18, and claims 19-28, which depend therefrom, no longer read on generating microorgans from synthetic scaffold.

The Examiner has further stated that the specification fails to enable generating microorgans by seeding a microorgan with differentiated cells. The Examiner cites a recent art publication (Liu, JBC, 2003), asserting that the state of the art at the time of filing indicated that differentiated cells have lost pluripotency and therefore the ability to generate multiple cell types. Applicant strongly disagrees with the Examiner's interpretation of the cited reference, and is of the opinion that the state of art at the time supports the feasability of the methods of the present invention for generating microorgans using differentiated cells .

Firstly, Liu, et al. clearly acknowledge the possibility of differentiated cells changing their supposed "final" cell fate:

> "While some of these results [transdifferentiation] can be explained by the presence of contaminating cell populations, persistence of pluripotent stem cells, cell fusion, etc., several examples exist that are difficult to explain as anything other than "true transdifferentiation" and/or dedifferentiation." (Liu, et al, JCB, 2003, abstract).

Such transdifferentiation was well known in the art at the time of filing. Tissue regeneration in injured or diseased organs, such as liver and retina is a well documented fact. Inducing differerentiated cells to change their phenotype has also been well documented. For example, US Patent Nos. 6,670,397 to Baranowitz, 6,087,168 to Levesque, et al, and 5,928,945 to Seliktar, et al. teach methods of effecting a change in the phenotype of non-stem cells, such as a fibroblast, liver cell or retinal cells, to achieve populations of cells capable of functioning distinct from the tissue from which the non-stem cells originated.

Further, and more importantly, Applicant wishes to stress that the methods of the present invention do not require transdifferentiation or dedifferentiation of seeded cells. The ability of seeded differentiated cells to proliferate and grow in three dimensional scaffolds, and maintain functions characteristic to the organs from which they originated is well known for many tissue types, such as chondrocytes (Chang et al Zhongua Kou 2002;37: 246-8), bone tissue (Holy et al, J Biomed Mater Res. 2000;51: 376-82) and hepatic tissue (Glicklis et al 2000; 67:344-53) (see also Vacanti, US Patent No. 5,770,417). Thus, the methods of the present invention for generating microorgans can be employed to produce microorgans having specific tissue functions resulting from, among other factors, the type of cell seeded on the microorgan scaffold:

> "The tissue type and function of such a synthetic micro-organ can be determined by the (i) conditions of culturing; (ii) body tissue type into which the seeded scaffold is implanted; (iii) micro-organ tissue from

> which the scaffold was generated and/or (iv) type of cells seeded on the scaffold (stem, and/or differentiated cells)…"(page 24, lines 19-23).

Thus, according to the instant specification, dedifferentiation of seeded cells, per se, is neither implicitly nor explicitly required for the use of the methods of the present invention for seeding differentiated cell types onto acellular scaffolds, and one of skill in the art, provided the teachings of the present invention, would not require undue experimentation to generate artificaial microorgans using devitalized acellular three dimensional scaffolds and differentiated cells, as taugh in the instant specification.

In view of the abovementioned arguments and amendments, Applicant believes to have overcome the 112, first paragraph rejections.

35 U.S.C. § 112, Second paragraph Rejections

The Examiner has rejected claims 18-28 under 35 U.S.C. 112, second paragraph, for failing to point out and distinctly claim the subject matter of the invention. The Examiner's rejections are respectfully traversed. Claims 18 and 28 have now been amended.

The Examiner has rejected claim 18, and claims dependent therefrom, for failing to set forth a concrete definition of the term "microorgan".

Firstly, Applicant has now elected to amend claim 18 to include the limitation "…providing a devitalized, acellular three dimensional scaffold…". Further, Applicant wishes to point out that the instant specification sets for concrete definition of microorgans in the "Detailed Description" section (page 18, lines 17-21):

> "For purposes of this specification and the accompanying claims, the terms "micro-organs", "MC", and "MCs" refer to at least one, preferably a plurality of, explants of tissue which retain the basic cell-cell, cell-matrix and cell-stroma architecture of the originating tissue. These terms refer to isolated as well as cultured explants."

The exact dimensions defining the microorgans of the present invention are also clearly set out in the paragraphs following the definition of "microorgans":

> "...cells positioned deepest within an individual micro-organ culture or explant are at least about 100 micrometers and not more than about 225 micrometers away from a nearest surface of the individual micro-organ culture..."(page 20, lines 15-18, and Table 1, page 21).

Thus, a "microorgan" is a tissue or organ explant of defined character, originating from a devitalized, acellular scaffold, and not, as asserted by the Examiner, "an organ portion of unique characters". Preparation of microorgans is clearly set out in the instant specification (see Materials and Experimental Methods page 28).

The Examiner has further stated that the metes and bounds of the term "repopulate" in step (b) of claim 18 are unclear. In order to expedite the prosecution of this case, and to further clarify the methods of the present invention, Applicant has elected to amend claim 18 to recite:

> "(b) seeding said acellular three dimensional scaffold with cells; and
>
> (c) providing conditions for cell growth and proliferation."

Conditions for cell growth and proliferation are well known in the art. Further, such conditions are described in detail in the present specification (see page 34, and Fig. 1 of the instant specification). It will be appreciated that conditions for cell proliferation and growth will vary according to the type of cell seeded on the devitalized, acellular scaffold.

Thus, now amended claim 18 no longer recites the term "repopulate".

The Examiner has rejected claim 28, stating that there is insufficient antecedent basis for the limitation of "said stem cells" in said claim. Claim 28 has now been amended to recite: "...said cells...", deriving antecedent basis from the "cells" recited in steps (a) and (b) of claim 18.

In view of the abovementioned arguments and amendments, Applicant believes to have overcome the 112, second paragraph rejections.

35 U.S.C. § 102 Rejections –Vacanti (1999, US Patent No. 5,855,610), Vacanti (1998, US Patent No. 5,770,417) and Riviere (1995, PNAS, 92:6733-37)

The Examiner has rejected claims 18-24 under 35 USC § 102(b) as being anticipated by Vacanti (1999, US Patent No. 5,855,610) (Vacanti I) and claim 18 as being anticipated by Vacanti (1998, US Patent No. 5,770,417)(Vacanti II). The Examiner's rejections are respectfully traversed. Claims 18 and and 28 have now been amended.

The Examiner has stated that Vacanti I and Vacanti II teach seeding a synthetic matrix or scaffold with various cell types, the scaffold having a 100-300 micron interstitial spacing limit, and that this limitation does not differ substantially from the 225-300 micron limit taught in the instant specification.

Applicant wishes to point out that Vacanti I and Vacanti II both relate to methods for the preparation of synthetic matrices for implantation of engineered tissues, and the matrices produced therefrom, for the generation of vascularized tissue in vivo. Vacanti I discloses the use of polyanhydride and polyorthoester polymer matrices for culturing of liver and intestinal cells before implantation (columns 7 and 8). Vacanti II discloses the seeding of periosteal cells, osteoblasts, chondrocytes, endothelial cells, smooth muscle cells, fibroblasts or skeletal muscle cells on polyglycolic acid scaffolds in tissue culture, and implanting the populated constructs into a recipient tissue (columns 7-10, Examples 1-5). In Vacanti II, the constructs were formed into tubular and lamellar structures to simulate vascular and osseous tissue, respectively. No mention is made, in any of the Examples, of the dimensions of the matrices used.

In stark contrast, the microorgans of present invention are generated using three dimensional scaffolds whose hallmark characteristic is the strict limitations of dimensions, such that "no cell is farther than about 225 micrometers from the neareast surface of microorgan culture, or closer than 100 micrometers from the source of gas and nutrients". Such microorgans differ fundamentally from the synthetic structures taught in Vacanti I and II.

The abovementioned notwithstanding, Applicant has elected to amend independent claim 18 to include the limitation of "providing a devitalized, acellular three dimensional scaffold...". Thus, independent claim 18, and claims 19-28, which depend therefrom, do not now read on generating microorgans from synthetic scaffolds. As such, it is Applicant's strong opinion that the synthetic constructs taught by Vacanti I and Vacanti II do not anticipate nor render obvious the methods of generating microorgans of the present invention as now claimed.

The Examiner has further rejected claims 18-20 and 22-28 under 35 USC 102(b) as being anticipated by Riviere (1995, PNAS, 92:6733-37). The Examiner's rejections are respectfully traversed. Claims 18 and 28 have now been amended.

The Examiner has stated that Riviere teaches transplanting bone marrow cells into lethally irradiated mice, and that the lethal irradiation produces, within the medullary space, an acellular scaffold comprising extracellular matrix. Applicant strongly disagrees with Examiner's contention.

Lethal radiation, exceeding 2 or 3 Grays, is used in myeloablative therapy for diseases of blood cell formation, such as leukemia, due to the sensitivity of rapidly dividing cells, such as the hematopoietic cells of the bone marrow, to radiation damage. The hematopoietic syndrome that follows such high doses of radiation is most always fatal, characterized by breakdown of hematopoiesis, and the ensuing systemic failure.

However, this is not to be interpreted to mean that the bone marrow is devitalized, and rendered acellular by high, lethal doses of radiation. In a recent review of the subject, Daniak et al (Hematology, 2003 (1): 473-96) noted that "The upper dose limit that can be survived without hematopoietic SCT might be in the range of 7–8 Gy with prompt use of hematopoietic factors." (page 487), indicating that a portion of the sensitive hematopoietic cells of the bone marrow survive even high doses of radiation. The existence of radioresistant bone marrow cells has been repeatedly demonstrated, both in vivo (see Inoue et al, Exp. Hematol 1995;23:1296-300, abstract enclosed) and in vitro (Kreja et at, Exp. Hematol. 1991;19:755-58, abstract enclosed), with D0's for CFU-S and BFU-E in excess of the 11Gy used by Riviere.

Further, it is well known in the art, that many of the non-hematopoietic cell

types found in bone marrow and stroma are not rapidly dividing cells, and by nature, significantly less susceptible to radiation effects. There is, then, no evidence that "lethal irradiation kills cells in the bone marrow...such that an acellular scaffold...remains."

Thus, one of skill in the art, using lethal irradiation to produce an acellular three dimensional scaffold for seeding with cells according to the teachings of the present invention, would fail to achieve the intended goal of generating microorgans. Thus, contrary to the Examiner's assertion, the method of lethal irradiation and bone marrow transplantation of Riviere does not, and cannot anticipate the method of the present invention, as taught in claims 18-20 and 22-28.

35 U.S.C. § 103(a) Rejections – Mitchell (US2002/0115208, August 2002) or Bruchman (US Patent No. 5,879,383, March 1999), in view of Vacanti (1999, US Patent No. 5,855,610) or Vacanti (1998, US Patent No. 5,770,417)

1) The Examiner has rejected claims 18-24 under 35 USC § 103(a) as being unpatentable over Mitchell (US2002/0115208, August 2002), in view of Vacanti (1999, US Patent No. 5,855,610)(Vacanti I) and Vacanti (1998, US Patent No. 5,770,417)(Vacanti II). The Examiner's rejections are respectfully traversed. Claims 18 and 28 have now been amended.

The Examiner has stated that Mitchell teaches decellularizing a three dimensional tissue, and seeding the remaining matrix with cells, and that Vacanti teaches interstitial size limitations for the scaffold between 100-300 microns, concluding that it would have been obvious for one of ordinary skill in the art to seed a scaffold as taught by Mitchell using size restrictions as taught by Vacanti, and that one of ordinary skill in the art would have been motivated to generate tissues in vitro using the size limitations because one would increase the likelihood of in vitro survival prior to implantation.

Applicant would like to point out that Vacanti I and II teach the production of synthetic scaffolds for culturing of cells before implantation. As noted by the Examiner, Vacanti does not teach using devitalized acellular three

dimensional scaffolds for generating engineered tissue. Whereas the process of preparing synthetic scaffolds for seeding cells in vitro is entirely different from the methods of preparing devitalized, acellular tissue explants having the dimensions and characteristics of microorgans, as taught in the present invention, it is Applicant's strong opinion that, contrary to the Examiner's assertion, Vacanti I and II would not motivate one of ordinary skill in the art to generate tissues from devitalized explants in vitro using the indicated size limitations.

That Vacanti I and II do not motivate for the use of such interstitial tissue size limitations is evident from the absence of any patents or patent applications in which such a combination of devitalized, acellular matrices and such interstitial size limitations are taught. For example, Vacanti I and/or II are cited in US Patent Nos. 6,753,181; 6,652,583; 6,497,725; 6,479,064; 6,438,802; 6,376,244; 6,206,917 all of which teach methods for decellularization of tissue and the generation of artificial organs therefrom, US Patent Nos. 6,783,776; 6,576,265; 6,521,750; 6,444,803; 6,300,127; 6,183,737; 6,110,480; 6,027,743; and 5,906,827, which teach devitalized tissue and the generation of artificial organs, and US Patent Application Nos. 0040044403; 0030215945; 0030194802; 0030180268; 0030124099; 0020160510; 0020102727 which teach decellularization or decellularized tissue scaffolds. All of the abovementioned US Patents or Patent Applications are silent regarding the interstitial size limitations mentioned in Vacanti I or Vacanti II. Rather, reference is made, on the whole, to the materials suitable for fashioning synthetic scaffolds taught in Vacanti I and II. No searchable patents or applications were uncovered relating to the interstitial size limitations mentioned in Vacanti I or II. Thus, although the limitations of interstitial size taught by Vacanti I and II were known to the authors of patents and applications relating to decellularized or devitalized acellular scaffolds, no

application of such knowledge is evident, strongly indicating the "nonobviousness" of such a combination.

Indeed, Mitchell cites Vacanti II in paragraphs 0081 and 0082, in reference to materials (substrates) and techniques suitable for establishing a three dimensional matrix for seeding with cells and culturing in vitro. However, Mitchell, similar to other inventors, fails to relate, in any manner, to the interstitial size limitations taught by Vacanti II, and teaches, in Example 5, culturing smooth muscle cells on a 2-3 centimeter segment of decellularized artery. In other examples, Mitchell further disregards Vacanti, teaching the decellularization of synthetic tissue engineered constructs intended for use as scaffolds, the portions being segments of engineered vessels no less than 2mm thick. One cannot assume that Mitchell would not have desired to use the size limitations taught by Vacanti to increase the likelihood of in vitro survival prior to implantation.

Thus, in view of the evidence that even experts in the field of decellularized, acellular three dimensional scaffolds such as Mitchell, familiar with the teachings of Vacanti, overlooked the interstitial size limitations taught therein, it is Applicant's strong opinion that one of ordinary skill in the art would not be motivated by Vacanti I or II in combination with the teachings of Mitchell to generate tissues from devitalized explants in vitro using the size limitations taught in the instant specification.

2) Likewise, the prior art reference Bruchman et al (US Patent No. 5,879,383), fails to teach or imply the importance of interstitial size limitations in the preparation and use of devitalized acellular three dimensional scaffolds for in vitro culture of engineered tissue constructs. Bruchman et al teach the preparation of a subendothelial matrix seeded with endothelial cells, for support of engineered vascular prostheses. Typically, devitalized tissue portions of 2.5 mm in diameter, and 2.5 cm in length are described, sleeved with GORE-TEX

supports, seeded with endothelial cells and cultured until engraftment, whole, into recipient animals (see Examples 1and 2).

Thus, one of ordinary skill in posession of the teachings of either Bruchman et al, or Mitchell, would not be motivated to combine these with the teachings of Vacanti I or Vacanti II, to generate tissues from devitalized explants in vitro using the size limitations taught in the instant specification, and as such it is the Applicant's strong opinion that the methods of the present invention for generating microorgans are neither anticipated nor rendered obvious by the teachings of Mitchell or Bruchman in combination with Vacanti I or Vacanti II.

In view of the above amendments and remarks it is respectfully submitted that amended independent claim 18, and all claims which directly or indirectly depend therefrom are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

Sol Sheinbein
RegistrationNo. 25,457
Date: November 10, 2004.

Encl.
Two month's extension fee
References:
Kreja et al (abstract)
Inoue et al (abstract)

1: Exp Hematol. 1991 Sep;19(8):755-8. Related Articles, Books, LinkOut

In vitro studies on the radiosensitivity of multipotent hemopoietic progenitors in canine bone marrow.

Kreja L, Weinsheimer W, Nothdurft W.

Institute for Occupational and Social Medicine, University of Ulm, Federal Republic of Germany.

The in vitro radiation response to 280-kV x-rays (does rate 72 cGy/min) of multipotent hemopoietic progenitor cells, mixed colony-forming units (CFU-mix), from canine bone marrow was assayed and compared to the radiation response characteristics of early erythroid progenitors, erythroid burst-forming units (BFU-E). To improve the colony-forming efficiency, the effect of various bone marrow cell separation techniques on colony formation of both progenitors was examined. The separation of bone marrow aspirates by discontinuous buoyant gradient centrifugation using the lymphocyte separation medium Lymphoprep with a density of 1.070 g/ml allowed the establishment of reproducible survival curves. The survival curves for both progenitors were strictly exponential, and CFU-mix were found to be more radiosensitive (D0 = 12 +/- 2 cGy) than BFU-E (D0 = 16 +/- 2 cGy).

PMID: 1868891 [PubMed - indexed for MEDLINE]

Help

1: Exp Hematol. 1995 Nov;23(12):1296-300. Related Articles, Books, LinkOut

Survival of spleen colony-forming units (CFU-S) of irradiated bone marrow cells in mice: evidence for the existence of a radioresistant subfraction.

Inoue T, Hirabayashi Y, Mitsui H, Sasaki H, Cronkite EP, Bullis JE Jr, Bond VP, Yoshida K.

Department of Pathology, Yokohama City University School of Medicine, Japan.

Because of increasing evidence of heterogeneity in the hematopoietic stem cell compartments, the radiosensitivity of spleen colony-forming units (CFU-S) was reevaluated to ascertain whether the classical single exponential curve for a graded dose of radiation is applicable at higher doses of radiation, 400-600 cGy. Bone marrow cells (BMC) removed from mice immediately after death under anesthesia were irradiated in vitro. Great care was taken to exclude anoxic effects during irradiation and to avoid any possible effects in the recipient mice from injection of excessive numbers of BMC. By estimating the number of cells to be injected to produce numbers of colonies within the evaluation range of the assay, we obtained a radiation survival curve that appeared to have a multiphasic concave shape; the D0 value for the 400-600 cGy range was estimated to be about 275 cGy, whereas the D0 for the lower doses was 95 cGy, the same value as previously reported. The reason a single exponential survival curve was previously obtained after graded doses of radiation is discussed, and a comparison of those results with the present data from in vitro radiation is made. Lacking experimental evidence, we speculate that the major factor that determines the slope of the survival curve is the degree to which the stem cells are in their normal hematopoietic environment during the irradiation. The probable existence of a fraction surviving after an exposure to 600 cGy, estimated by the limiting dilution assay, was about 1 per 2 x 10(6) BMC. Such radio-insensitive CFU-S appear to be primitive CFU-S, which can contribute materially to the long-term survival of lethally irradiated bone marrow recipients.

PMID: 7589285 [PubMed - indexed for MEDLINE]

Help